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Elhissi, A., Brar, J., Najlah, M., Roberts, S., Faheem, A., & Taylor, K. (2013). An Ethanol-Based Proliposome Technology for Enhanced Delivery and Improved Respirability of Antiasthma Aerosols Generated Using a Micropump Vibrating-Mesh Nebulizer. *Journal of Pharmaceutical Technology, Research and Management*, 1(2), 171-180.

[Link to publication record in Ulster University Research Portal](#)

### **Published in:**

Journal of Pharmaceutical Technology, Research and Management

### **Publication Status:**

Published (in print/issue): 01/11/2013

### **Document Version**

Publisher's PDF, also known as Version of record

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# An Ethanol-Based Proliposome Technology for Enhanced Delivery and Improved “Respirability” of Antiasthma Aerosols Generated Using a Micropump Vibrating-Mesh Nebulizer

ABDELBARY M.A. ELHISSI<sup>1,2\*</sup>, JASMEET BRAR<sup>3</sup>,  
MOHAMMAD NAJLAH<sup>4</sup>, SIMON A. ROBERTS<sup>3</sup>,  
AHMED FAHEEM<sup>5</sup>, KEVIN M.G. TAYLOR<sup>1,3</sup>

<sup>1</sup>Institute of Nanotechnology and Bioengineering, <sup>2</sup>School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston PR1 2HE, England, United Kingdom

<sup>3</sup>Department of Pharmaceutics, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, England, United Kingdom

<sup>4</sup>Faculty of Pharmacy, Albaath University, Homs, Syrian Arab Republic

<sup>5</sup>School of Pharmacy and Pharmaceutical Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland, UK

Email: aelhissi@yahoo.com or aelhissi@uclan.ac.uk

**Abstract** Salbutamol sulphate liposomes were generated using ethanol-based proliposomes followed by nebulization using an Aeroneb Pro vibrating-mesh nebulizer. The droplet size, output and fine particle fraction (FPF) of the drug incorporated in liposome formulation were compared to those of a conventional drug solution. Aerosol output was determined gravimetrically and drug output was analyzed by using high performance liquid chromatography. The potential of aerosol deposition in deep lung was evaluated using inertial impaction and laser diffraction. The effect of formulation surface tension on the aerosol performance was studied. Output and FPF were improved using liposomes compared to the conventional solution, for instance, FPF values were 57.85% and 45.81% respectively. The volume median diameter as measured by laser diffraction was respectively 3.44  $\mu\text{m}$  and 3.22  $\mu\text{m}$ ; however, the higher FPF of the liposome formulation is justified by the lower polydispersity of its aerosol. The improved aerosol performance using liposomes was attributed to the reduction of surface tension caused by the presence of phospholipid. This is the first study that demonstrates the ability of liposomes to improve the nebulized drug output and FPF.

**Keywords:** Formulation, Inhalation, Lung, Pulmonary, Solution

Journal of Pharmaceutical  
Technology, Research and  
Management  
Volume 1, No. 2,  
November 2013  
pp. 171–180



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## 1. INTRODUCTION

Liposomes are phospholipid carrier vesicles that are made of materials which are very similar to the components of lung surfactants. Many studies have demonstrated that liposomes are generally safe for pulmonary delivery (Kellaway and Farr, 1990). Therapeutically, liposomes can prolong the action of the entrapped drug in the respiratory tract; hence they can potentially improve the therapeutic outcome and reduce the systemic adverse effects of the drug (Taylor et al., 1989; Saari et al., 1999; Weers et al., 2009). The main limitation of liposomes is their instability in aqueous dispersions, since phospholipids are liable to hydrolysis (Kensil and Dennis, 1981) and oxidation (Hunt and Tsang, 1981); this may cause the liposomes to aggregate or fuse, resulting in leakage of the drug originally entrapped.

Freeze-drying (lyophilization) in the presence of suitable cryoprotectants has been used to resolve the problem of liposome instabilities (Gordon et al., 1982; van Bommel and Crommelin, 1984; Crowe et al., 1986). Recently, we have shown that two antiasthma drugs can be included in small unilamellar liposome formulations using freeze-drying with sucrose or trehalose as cryoprotectants (Elhissi et al., 2010). Proliposome technologies are economical alternatives to freeze-drying. For instance, ethanol-based proliposomes are concentrated ethanolic solutions of phospholipid which generate liposomes by addition of aqueous phase and shaking (Perrett et al., 1991). The suitability of ethanol-based proliposomes to generate inhalable liposomes via nebulizers has been demonstrated (Elhissi et al., 2006; Elhissi et al., 20011).

The therapeutic benefit of aerosols in pulmonary drug delivery is determined by the percentage of dose delivered in the “fine particle fraction” (FPF); the dose fraction which is delivered in aerosol particles smaller than 5-6  $\mu\text{m}$  and is considered “respirable” and hence likely to reach the peripheral airways (i.e. bronchioles and alveolar region) (Stahhofen et al., 1980; O’Callaghan and Barry, 1997).

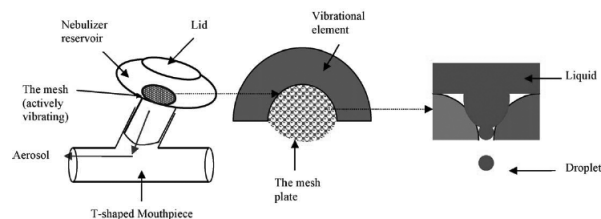
Pulmonary delivery of “respirable” liposome aerosols is well-established using medical nebulizers (Knight and Gilbert, 1988; Taylor et al., 1989; Saari et al., 1999). Currently, there are three types of nebulizers: air-jet, ultrasonic and vibrating-mesh nebulizers (Elhissi and Ahmed, 2011). Whilst traditional ultrasonic nebulizers are generally unsuitable for delivery of liposomes (Elhissi and Taylor, 2005), air-jet nebulizers are superior in delivery of “respirable” liposome aerosols (Farr et al., 1985; Waldrep et al., 1993; Waldrep et al., 1994; Saari et al., 1998; Saari et al., 1999; Albasarah et al., 2010). However, the shearing occurring within air-jet nebulizers may damage the liposome bilayers, resulting in considerable losses of entrapped hydrophilic materials (Taylor et al., 1990; Elhissi et al., 2007). The more recently commercialized

type of nebulizers, namely vibrating-mesh nebulizers (Dhand, 2002; Elhissi and Ahmed, 2011) are suitable for liposome delivery (Elhissi and Taylor, 2005; Li et al., 2008; Gaspar et al., 2010; Elhissi et al., 2011; Elhissi et al., 2012) and may cause less losses of the entrapped drug during aerosolization when compared to air-jet nebulizers (Elhissi et al., 2006; Elhissi et al., 2007; Kleemann et al., 2007).

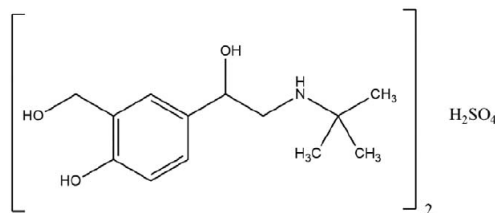
An example of vibrating-mesh nebulizers is the Aeroneb Pro device (Fig.1) which employs a perforated plate that is connected to a vibrational element, causing a “micropump” action which extrudes the medical fluid and generates the aerosol (Dhand, 2002; Ghazanfari et al., 2007). The aerosol properties of vibrating-mesh nebulizers are highly influenced by fluid physicochemical properties when conventional solutions are nebulized (Ghazanfari et al., 2007; Najlah et al., 2013).

Salbutamol sulphate (Fig.2) is a selective short acting  $B_2$ -adrenoceptor agonist that is widely used for the treatment of asthma. Using liposomes generated from ethanol-based proliposomes, the entrapment efficiency of this drug in liposomes may exceed 60% (Elhissi et al., 2006; Elhissi et al., 2011). In this study, the aerosol properties of salbutamol sulphate liposomes generated from proliposomes were compared to aerosols generated from a conventional drug solution using the micropump Aeroneb Pro vibrating-mesh nebulizer. This is the first study that evaluates the effect of liposomes on the aerosol properties of nebulizers in terms of output and FPF.

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**Figure 1.** Design of the Aeroneb Pro vibrating-mesh nebulizer (Ghazanfari et al., 2007)



**Figure 2.** Chemical structure of salbutamol sulphate

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## 2. MATERIALS AND METHODOLOGY

### 2.1 Materials

Soya phosphatidylcholine (SPC; Lipoid S-100) was a gift from Lipoid, Switzerland. Salbutamol sulphate was purchased from Alfa Aesar, UK. Sodium chloride (NaCl) and absolute ethanol were of analytical grade “AnalaR” and supplied by VWR, UK. Cholesterol (99%), sodium hexane sulphonate, Triton X-100 were purchased from Sigma Aldrich, UK. Water and methanol used in high performance liquid chromatography (HPLC) were of HPLC grade and supplied by Fisher Scientific Ltd., UK. The Aeroneb Pro micropump nebulizer was supplied as a gift by Aerogen Ltd, Ireland.

### 2.2 Methods

#### 2.2.1 Preparation of liposomes

The lipid phase (50 mg) consisting of SPC and cholesterol (1:1) was dissolved in ethanol (60 mg) with heating for 1 min at 60°C. This quantity of ethanol was found to be appropriate to dissolve the lipids (Elhissi et al., 2006). Salbutamol sulphate in NaCl (0.9%) solution (5 mg/ 100 µl) was added and formulation was mixed for 1 min to generate a “milky” dispersion of liposomes. The preparation was further diluted with 4.9 ml drug-free solution of NaCl (0.9%). This formulation was prepared by following the procedure conducted in our previous publications, and found to yield an entrapment efficiency exceeding 60% for this drug (Elhissi et al., 2006; Elhissi et al., 2011). Surface tension measurements of liposome formulations and conventional salbutamol sulphate solution were performed using a Kibron Delta-8 multichannel microtensiometer (Kibron, Finland).

#### 2.2.2 Aerosol droplet size analysis

Droplet size and size distribution of aerosols generated with the Aeroneb Pro nebulizer were analyzed using the Malvern 2600c laser diffraction size analyzer (Malvern Instruments Ltd., UK) by recording the volume median diameter (VMD; 50% undersize) and Span values, respectively.  $\text{Span} = (90\% \text{ undersize} - 10\% \text{ undersize}) / \text{VMD}$ .

#### 2.2.3 Nebulizer output study and determination of FPF

A two-stage impinger was set up by placing 30 ml and 7 ml of NaCl (0.9%) solution in its lower and upper stages respectively. The flow rate through the

impinger was set up at 60 L/min. At this flow rate, the cut-off aerodynamic diameter between the upper and lower stages is 6.4  $\mu\text{m}$  (Hallworth and Westmoreland, 1987). The Aeroneb Pro nebulizer (Aerogen Ltd, Ireland) was accurately weighed and filled with liposome dispersion or drug solution (5 ml) and reweighed. The nebulizer was directed towards the “throat” of the impinger and nebulization was performed to “dryness” as determined by 30 seconds after complete cessation of the aerosol generation. The nebulizer was weighed for the third time and the aerosol output was calculated by calculating the weight difference. The drug output and distribution between the upper and lower stages of the impinger were determined using HPLC according to the method previously described by Elhissi et al. (2006). The drug output was multiplied by the fraction of the drug deposited in the lower stage of the impinger (i.e. drug in aerosol droplet  $<6.4 \mu\text{m}$ ) to calculate the FPF for each formulation.

#### 2.2.4 Statistical analysis

All experiments were conducted three times. Analysis of variance (ANOVA) and student’s t-tests were performed. When the calculated P value was less than 0.05 the difference between the compared groups was considered statistically significant.

### 3. RESULTS AND DISCUSSION

#### 3.1 Surface tension of formulations

Table 1 shows that surface tension is significantly ( $P < 0.05$ ) affected by formulation, with liposome formulations having much lower surface tension when compared to lipid-free formulations. The presence of salbutamol sulphate or sodium chloride did not affect the measured surface tension (Table 1),

**Table 1.** Surface tension measurements of formulations ( $n = 3 \pm \text{SD}$ ).

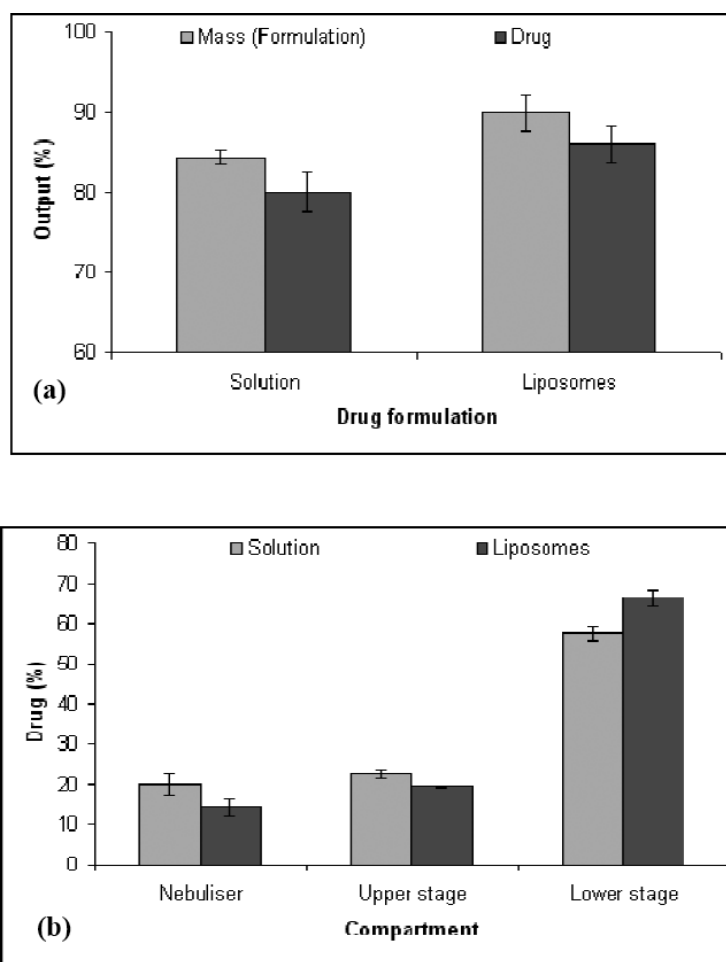
Formulation	Surface tension (mN/m)
Deionised water	$72.32 \pm 0.44$
NaCl (0.9%)	$72.09 \pm 0.44$
Drug solution	$72.10 \pm 0.57$
Liposomes (without drug)	$31.63 \pm 1.95$
Liposomes (with drug)	$31.38 \pm 1.97$

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indicating that the activity exerted on the surface by these salts was minimal and insignificant.

### 3.2 Nebulization performance study

The output study showed that both formulations had insignificantly ( $P>0.05$ ) higher mass output than drug output (Fig.3a). The similarity between drug



**Figure 3.** Aerosol mass and drug outputs from the Aeroneb Pro nebulizer (a) and drug distribution between the nebulizer reservoir and the impinger stages (b) using the conventional salbutamol sulphate solution and proliposome formulation ( $n = 3 \pm SD$ ).

output and aerosol output indicates that solvent evaporation from the nebulizer was minimal and the presence of liposomes did not hinder the delivery of the drug. By contrast, previous studies have demonstrated that air-jet nebulizers cause solvent evaporation during nebulization and concentrate the soluble material within nebulizer reservoir (McCallion et al., 1996). Aerosol mass output and drug output were significantly ( $P<0.05$ ) higher for the liposomal preparation compared to the conventional solution (Fig.3a), indicating that the inclusion of phospholipid was desirable, probably because phospholipid reduced the surface tension of formulation (Table 1). This clearly demonstrates a novel application of liposomes in nebulizer formulations.

Liposomes also significantly ( $P<0.05$ ) increased the fraction of drug delivered to the lower impinge stage and decreased the deposition in the upper stage of the impinger (Fig.3b). This suggests that nebulized liposomes may not only offer a sustained and localized drug effect in the respiratory airways (Taylor et al., 1989; Saari et al., 1999; Weers et al., 2009) but can also increase the dose delivered in “respirable” fraction. Thus, liposomes may play roles beyond entrapment of drugs when the Aeroneb Pro vibrating-mesh nebulizer is employed, with inclusion of lipid having a desirable effect on drug delivery and deposition in the “FPF”.

The liposomal preparation produced aerosol droplets of larger VMD ( $P<0.05$ ) and smaller Span ( $P<0.05$ ) (Table 2). Thus, the higher deposition in the lower stage impinger of the liposomal formulation (Fig.3b) was attributed to the narrower size distribution (i.e. lower polydispersity) of the aerosols when phospholipid was included within formulations (Table 2). This was attributed to the lower surface tension of the fluid as a result of lipid inclusion (Table 1). Using Newtonian fluids, the droplet size and size distribution of aerosols generated using vibrating-mesh nebulizers were shown to be highly affected by the fluid physicochemical properties such as viscosity, surface tension (Ghazanfari et al., 2007) and inclusion of ions (Ghazanfari et al., 2007; Najlah et al., 2013).

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**Table 2.** Size and size distribution of aerosol droplets after 5 min nebulization using laser diffraction, and FPF as calculated using the two-stage impinger at the end of nebulization ( $n = 3 \pm \text{SD}$ ).

Formulation	VMD ( $\mu\text{m}$ )	Span	FPF (%)
Solution	$3.22 \pm 0.02$	$2.16 \pm 0.04$	$45.81 \pm 1.03$
Liposomes	$3.44 \pm 0.05$	$1.98 \pm 0.05$	$57.85 \pm 0.75$



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#### 4. CONCLUSION

This study has shown that an ethanol-based approach to generating liposomes can be used to prepare a salbutamol sulphate formulation with enhanced drug output and improved “FPF” compared to a conventional solution of the drug when nebulization was performed using the micropump Aeroneb Pro vibrating-mesh nebulizer.

#### 5. ACKNOWLEDGEMENTS

We thank Lipoid, Switzerland for supplying us with the SPC (Lipoid S-100) and Aerogen, Ireland for supplying us with the Aeroneb Pro nebulizer.

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